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Plant Gene Register

Nucleotide Sequence of a cDNA for the Potato (*Solanum tuberosum* L.) Chloroplast Ribosomal Protein S16[1]

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Plant chloroplast genes encode rRNAs, tRNAs, and proteins for their own transcription and translation systems. Plant chloroplast ribosomes, which are a part of the translational apparatus, are prokaryotic like, 70S in size, and comprise 16S, 23S, 5S, and 4.5S rRNAs and about 58 to 62 different ribosomal protein species (Subramanian, 1985). Nineteen to 20 different ribosomal protein genes that code 11 or 12 proteins of the small ribosomal subunit and 8 proteins of the large subunit are located in the chloroplast genome (Shinozaki et al., 1986b).

Here we report the full-length sequence of a cDNA encoding the potato (*Solanum tuberosum* L.) chloroplast ribosomal protein S16 (cp *rps16*) (Table I). The deduced amino acid sequence of cp *rps16* gene is over 80% conserved among higher plants, but is less than 30% conserved between plants and *Escherichia coli* (Kanakari et al., 1992). Stern et al. (1988) reported that *E. coli* ribosomal protein S16 was associated with other ribosomal proteins (S4, S8, and S20) to facilitate the binding and assembly of the 16S rRNA into the 30S subunit.

Using conventional differential screening techniques, we obtained several early tuber cDNA clones from a 4-d axillary bud-tuber λZAP library. One of these clones, designated p24–2, contained an approximately 1.6-kb insert and was sequenced in its entirety. The 1561-bp sequence showed high homology with known chloroplast *rps16* genes, which code for chloroplast ribosomal protein S16 of tobacco, rice, mustard, and maize (Shinozaki et al., 1986a, 1986b; Hiratsuka et al., 1989; Neuhaus et al., 1989; Kanakari et al., 1992, respectively). Based on the DNA sequence similarity and its amino acid sequence analysis, we concluded that the cDNA p24–2 was the potato chloroplast *rps16* gene. This potato cp *rps16* gene is located between the *trnK* gene for $tRNA^{Lys}$ (UUU) and the *trnQ* gene for $tRNA^{Gln}$ (UUG) on the same strand in the chloroplast genome. The 5′ untranslated sequence contains an AT-rich region of 239 nucleotides and a putative ribosome-binding sequence element 5′-AGGA-3′ that is located five nucleotides upstream from the initiation codon, ATG. Two reading

Table I. *Characteristics of a cDNA for potato chloroplast rps S16*

Organism:
Potato (*Solanum tuberosum* L. cv Superior).

Genome Location:
Between the *trnK* gene for $tRNA^{Lys}$ (UUU) and *trnQ* gene for $tRNA^{Gln}$ (UUG) on the same strand in the chloroplast genome.

Gene Product:
Chloroplast ribosomal protein S16 (cp RPS16).

Source:
cDNA library in λZAPII constructed from poly(A)$^+$ RNA isolated from 4-d axillary bud tuber. It is proposed that the 3′ end containing several inverted repeat sequences allowed cDNA synthesis.

Techniques:
Differential screening techniques, restriction fragment subcloning into pGEM11Z, dideoxy sequencing of both strands of overlapped fragments.

Methods of Identification:
Comparison of the published cp *rps16* gene and its amino acid-deduced sequences of tobacco (Shinozaki et al., 1986a), mustard (Neuhaus et al., 1989), and maize (Kanakari et al., 1992).

Features of the cDNA Structure:
Total length of 1561 bp, representing a full-length unprocessed transcript containing a group II intron of 855 bp, exon 1 of 40 bp, exon 2 of 227 bp, AT-rich 239 bp of the 5′ untranslated sequence containing a ribosomal binding sequence element 5′-AGGA-3′, a 3′ untranslated region of 146 bp containing five inverted repeat sequences. No poly(A)$^+$ tail was observed.

Features and Function of the Deduced Amino Acid Sequence:
Two exons encode an 88-amino acid sequence of RPS16. The 13 amino acids encoded by exon 1 are highly conserved among higher plants. Homology of the cp RPS16 to other plants showed 95% with tobacco, 90% with maize, 41% with *Cyanidium caldarium*, and 33% with *E. coli*. Function of RPS16 is associated with ribosomal proteins S4, S8, and S20 to facilitate the binding and assembly of the 16S rRNA into the 30S subunit (Stern et al., 1988).

Subcellular Location:
Chloroplast.

Expression Characteristics:
Two transcripts, about 1.5-kb immature and 0.7-kb mature RNAs, have been identified in chloroplast. The expression is developmentally regulated with transcript levels decreasing during tuberization and increasing during shoot and leaf growth. Accumulation of S16 transcripts is constitutive in proplastids, reduced in amyloplasts, and enhanced by light in chloroplasts.

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Abbreviations: cp, chloroplast; *rps*, ribosomal protein small subunit.

frames are interrupted by an intron of 855 nucleotides. Exon 1 is 40 bp long and extends from the nucleotide position 294 to position 333 and encodes 13 amino acids. Exon 2 consists of 227 bp, encodes 75 amino acids, and is located from the nucleotide position 1189 to a termination codon TAA at position 1415. The intron interrupts the CGA Arg codon between C and G. No putative open reading frame is contained in the intron.

This structure is similar to that found in tobacco (Shinozaki et al., 1986a) and in mustard cp *rps16* (Neuhaus et al., 1989). The 5′ end, 5′-GTGCGACTTG-3′, and the 3′ end, 5′-TCTATCCCAAT-3′, of the intron boundary sequences of the potato cp *rps16* are highly conserved among mustard, maize, and tobacco. Both the 5′ and 3′ boundary sequences of the potato cp *rps16* intron showed typical characteristics of related intron types of group II, including introns of tobacco cp *rps12*, *rpl2*, and *rpl16* genes (Ohto et al., 1988). The 3′ end of the untranslated sequence consists of 146 bp and shows typical plastid gene construction in that it contains no polyadenylation signal, but instead has several inverted repeat sequences, which could form a hairpin structure in their DNA and a stem-loop structure in their transcripts. The inverted repeats include one 5′ to 3′ direction element 5′-GGAAATACAAAAAA-3′ with a completely inverted complement of this sequence, and three different sets of inverted direction elements. The putative stem-loop structure created by 3′ inverted repeats is typical of plastid mRNA. These inverted repeat sequences resemble prokaryotic terminators and may function as termination signals during chloroplast transcription (Sijben-Müller et al., 1986).

In contrast, it was suggested that the 3′ inverted repeat elements of other chloroplast genes were ineffective as transcription terminators, but instead functioned to increase the stability of chloroplast transcripts (Stern and Gruissem, 1987). The potato cp *rps16* gene has properties of both prokaryotic [Shine-Dalgarno element, no poly(A^+) sequence] and eukaryotic (intron sequence) gene structure.

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LITERATURE CITED

Hiratsuka S, Shimada H, Whitter R, Ishibashi I, Sakamoto M, Mori M, Kondo C, Honji Y, Sun C-R, Meng B-Y, Li Y-O, Kanno A, Nishizawa Y, Hirai A, Shinozaki K, Sugiura M (1989) The complete sequence of rice (*Oryza sativa*) chloroplast genome: intermolecular recombination between distinct tRNA genes accounts for a major plastid DNA inversion during the evolution of the cereals. Mol Gen Genet **217:** 185–194

Kanakari S, Timmler G, von Knoblauch K, Subramanian AR (1992) Nucleotide sequence, map position and transcript pattern of the intron-containing gene for maize chloroplast ribosomal protein S16. Plant Mol Biol **18:** 419–422

Neuhaus H, Scholz A, Link G (1989) Structure and expression of a split chloroplast gene from mustard (*Sinapis alba*): ribosomal protein *rps16* reveals unusual transcriptional features and complex RNA maturation. Curr Genet **15:** 63–70

Ohto C, Torazawa K, Tanaka M, Shinozaki K, Sugiura M (1988) Transcription of ten ribosomal protein genes from tobacco chloroplasts: a compilation of ribosomal protein genes found in the tobacco chloroplast genome. Plant Mol Biol **11:** 589–600

Shinozaki K, Deno H, Sugita M, Kuramitsu S, Sugiura M (1986a) Intron in the gene for the ribosomal protein S16 of tobacco chloroplast and its conserved boundary sequences. Mol Gen Genet **202:** 1–5

Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chunwongse J, Obokata J, Yamaguchi-Shinozaki K, Ohto C, Torazawa K, Meng BY, Sugita M, Deno H, Kamogashira T, Yamada K, Kusuda J, Takaiwa F, Kato A, Tohdoh N, Shimada H, Sugiura M (1986b) The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J **5:** 2043–2049

Sijben-Müller G, Hallick RB, Alt J, Westhoff P, Herrmann RG (1986) Spinach plastid genes coding for initiation factor IF-1, ribosomal protein S11 and RNA polymerase α-subunit. Nucleic Acids Res **14:** 1029–1045

Stern DB, Gruissem W (1987) Control of plastid gene expression: 3′ inverted repeats act as mRNA processing and stabilizing elements, but do not terminate transcription. Cell **51:** 1145–1157

Stern S, Changchien L-M, Craven GR, Noller HF (1988) Interaction of proteins S16, S17 and S20 with 16S ribosomal RNA. J Mol Biol **200:** 291–299

Subramanian AR (1985) The ribosome: its evolutionary diversity and the functional role of one of its components. Essays Biochem **21:** 45–85